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(54) Title: MYT1 KINASE INHIBITORS (57) Abstract Novel myt1 kinase receptor antagonists and methods of using them are provided.		

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MYT1 KINASE INHIBITORS

FIELD OF THE INVENTION

The present invention relates to membrane-associated tyrosine and threonine kinase ("myt1 kinase") enzyme inhibitors, pharmaceutical compositions comprising these compounds and methods for identifying these compounds and methods of using these compounds to treat various forms of cancer and hyperproliferative diseases.

BACKGROUND OF THE INVENTION

Entry into mitosis is initiated by the M phase-promoting factor (MPF), a complex containing the cdc2 protein kinase and cyclin B. Proper regulation of MPF ensures that mitosis occurs only after earlier phases of the cell cycle are complete. Phosphorylation of cdc2 at Tyr-15 and Thr-14 suppresses this activity during interphase (G1, S, and G2). At G2-M transition, cdc2 is dephosphorylated at Tyr-15 and Thr-14 allowing MPF to phosphorylate its mitotic substrates. A distinct family of cdc-regulatory kinases (Wee1) is known to be responsible for phosphorylation of the cdc Tyr-15. A new member of this family, Myt1 was recently described as the Thr-14 and Tyr-15-specific cdc2 kinase, and shown to be an important regulator of cdc2/cyclin B kinase activity (Science 270:86-90, 1995; Mol. Cell. Biol. vol 17:571, 1997). The inhibitory phosphorylation of cdc2 is important for the timing of entry into mitosis. Studies have shown that premature activation of cdc2 leads to mitotic catastrophe and cell death. Inhibition of Myt1 is predicted to cause premature activation of cdc2, and thus would kill rapidly proliferating cells. In addition, Myt1 inhibition is predicted to reduce resistance to conventional DNA-damaging chemotherapeutics, because the mechanisms by which cells avoid death involve arrest in the G2 phase of the cell cycle, and repair or DNA damage prior to division. That arrest should be prevented by blocking Myt1 inhibitory phosphorylation of cdc2. Thus forcing the cell to enter mitosis prematurely.

Myt1 kinase is an important cell cycle regulator, particularly at the G2/M phase. Inhibitors would therefore be attractive for the treatment of cancer. Current cancer therapies, including surgery, radiation, and chemotherapy, are often unsuccessful in curing the disease. The patient populations are large. For example, in colon cancer alone there are 160,000 new cases each year in the US, and 60,000 deaths. There are 600,000 new colon cancer cases each year worldwide. The number for lung cancer are twice that of colon cancer. The largest deficiency of chemotherapies for major solid tumors is that most

patients fail to respond. This is due to cell cycle regulation and subsequent repair of damage to DNA or mitotic apparatus, the targets for most effective chemotherapeutic agents. Myt1 kinase offers a point of intervention downstream from these mechanisms by which tumor cells develop resistance. Inhibition of Myt1 could in and of itself have therapeutic benefit in reducing tumor proliferation, and in addition, could be used in conjunction with conventional chemotherapies to overcome drug resistance.

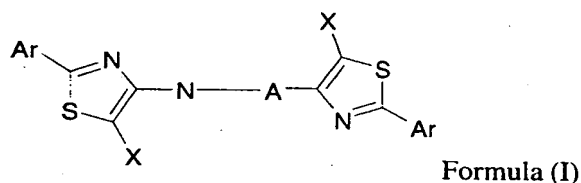
Based on the foregoing, there is a need to identify a potent myt1 kinase enzyme inhibitor for the treatment of various indications, including cancer, associated with the present receptor.

SUMMARY OF THE INVENTION

The present invention involves compounds represented by Formula (I) hereinbelow, pharmaceutical compositions comprising such compounds and methods of antagonizing the myt1 kinase receptor using these compounds.

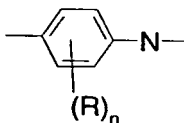
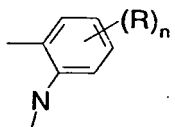
DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compounds of Formula (I), hereinbelow:

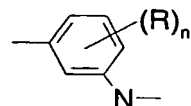


Formula (I)

wherein A represents a covalent bond, or a 1,2, 1,3 or 1,4-disubstituted aryl amine ring selected from the group consisting of:



, and



wherein

R is independently selected from the group consisting of H, OMe, Cl, Br, F, NO₂, and CN;

n is an integer from 1 to 4;

each X is independently selected from the group consisting of H, Br, CH₃, NO₂, CN, and NR₁R₂;

each Ar is independently optionally substituted phenyl or an optionally substituted 5 or 6
5 membered heterocyclic ring comprising one or more heteroatoms selected from N, O and S;
and

R₁ and R₂ are, independently, hydrogen or C₁₋₄ alkyl, branched or cyclic, optionally containing O or N.

Preferred compounds of the present invention are selected from the group
10 consisting of:

bis[2-(4-phenyl-5-methyl)thiazolyl]amine,

bis[2-(4-(2-pyridyl)thiazolyl]amine,

bis[2-[4-(3-pyridyl)thiazolyl]amine ,

N,N-Bis(5(3,4-dichlorophenyl)-2-thiazolyl)amine,

15 N,N-bis(4-(4'-biphenyl)-2-thiazolyl)amine,

1,4-bis(4-(4'-biphenyl)-2-thiazolylamino)benzene,

1,3-bis[4-(3-pyridyl)-2-thiazolylamino]benzene,

1,4-bis[4-fluorophenyl-2-thiazolylamino]benzene; and

1,4-bis(4-(4-methoxyphenyl)-2-thiazolylamino)benzene.

20 More preferred compounds of the present invention are selected from the group consisting of:

bis[2-(4-phenyl-5-methyl)thiazolyl]amine,

1,4-bis(4-(4-methoxyphenyl)-2-thiazolylamino)benzene,

N,N-bis(4-(4'-biphenyl)-2-thiazolyl)amine,

25 1,4-bis(4-(4'-biphenyl)-2-thiazolylamino)benzene,

1,4-bis[4-fluorophenyl-2-thiazolylamino]benzene,

bis[2-(4-(2-pyridyl)thiazolyl]amine and

bis[2-[4-(3-pyridyl)thiazolyl]amine.

The most preferred compounds of the present invention are selected from the group
30 consisting of:

bis[2-(4-phenyl-5-methyl)thiazolyl]amine,

1,4-bis(4-(4-methoxyphenyl)-2-thiazolylamino)benzene,

N,N-bis(4-(4'-biphenyl)-2-thiazolyl)amine, and

1,4-bis(4-(4'-biphenyl)-2-thiazolylamino)benzene hydrobromide.

As used herein, "alkyl" refers to an optionally substituted hydrocarbon group joined together by single carbon-carbon bonds. The alkyl hydrocarbon group may be linear, branched or cyclic, saturated or unsaturated. Preferably, the group is saturated linear or cyclic.

5 The compounds of the present invention may contain one or more asymmetric carbon atoms and may exist in racemic and optically active forms. All of these compounds and diastereomers are contemplated to be within the scope of the present invention.

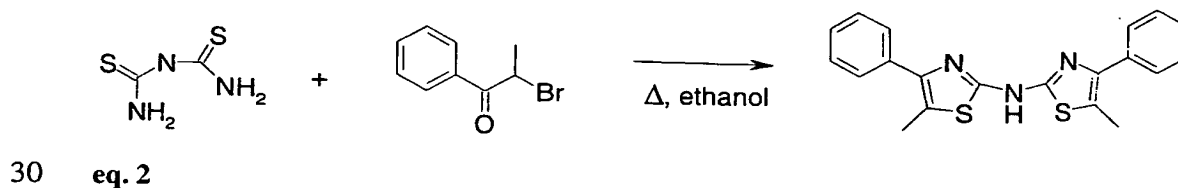
The present compounds can also be formulated as pharmaceutically acceptable salts and complexes thereof. Pharmaceutically acceptable salts are non-toxic salts in the
10 amounts and concentrations at which they are administered.

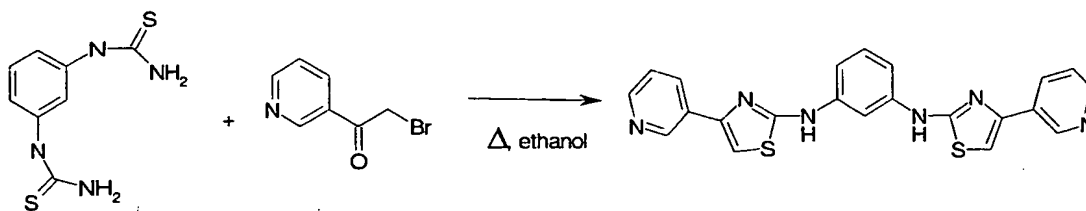
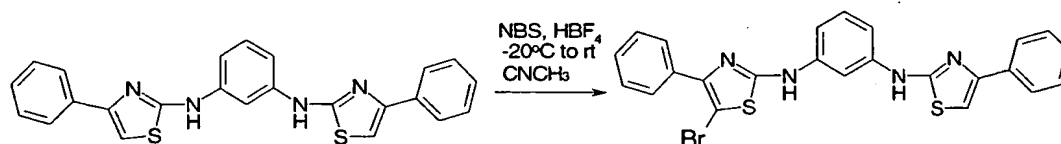
Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride, fumarate, maleate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, cyclohexylsulfamate and quinate. Pharmaceutically acceptable salts can be obtained from
15 acids such as hydrochloric acid, maleic acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, fumaric acid, and quinic acid.

Pharmaceutically acceptable salts also include basic addition salts such as those
20 containing benzathine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, aluminum, calcium, lithium, magnesium, potassium, sodium, ammonium, alkylamine, and zinc, when acidic functional groups, such as carboxylic acid or phenol are present.

Preferred salts include hydrobromide, dihydrobromide and bistrifluoroacetate.
25 The present compounds are readily prepared by the schemes represented below:

Scheme 1



**Scheme 2**

The corresponding bisthiazolyl compounds are synthesized via condensation of
 5 dithiobiuret (as in eq. 1) or any other bisthiourea such as 1,3-phenyldithiourea (as in eq.2)
 with two equivalents of an appropriately substituted bromoketone such as 2-bromo-1-
 phenylpropan-1-one in a solvent such as ethanol or acetone or any other suitable solvent.
 The reaction mixture is heated to 70-90°C for several hours (4-12 h). Cooling of the
 reaction yields the desired product as a bis-hydrobromide salts.

10 Monobromination of the bisthiazole compounds can be accomplished for example via the
 reaction of N-bromosuccinimide in the presence of an acid such as Fluoroboric acid, or any
 other suitable protic acid, in a solvent such as acetonitrile or any other suitable solvent,
 following a procedure described in the literature (Oberhauser, T. *J.Org. Chem.*, **1997**, 62,
 4504-4506). Bis-bromination can be accomplished via any known bromination reaction, such
 15 as reaction of the bis-thiazole with N-bromosuccinimide using in refluxing DMSO or
 acetonitrile or Bromine in acetic acid or any other method known to chemists skilled in the
 art.

With appropriate manipulation and protection of any chemical functionality,
 synthesis of the remaining compounds of Formula (I) is accomplished by methods
 20 analogous to those above.

In order to use a compound of Formula (I) or a pharmaceutically acceptable salt
 thereof for the treatment of humans and other mammals, it is normally formulated in
 accordance with standard pharmaceutical practice as a pharmaceutical composition.

25 The present ligands can be administered by different routes including intravenous,
 intraperitoneal, subcutaneous, intramuscular, oral, topical, transdermal, or transmucosal
 administration. For systemic administration, oral administration is preferred. For oral
 administration, for example, the compounds can be formulated into conventional oral

dosage forms such as capsules, tablets and liquid preparations such as syrups, elixirs and concentrated drops.

Alternatively, injection (parenteral administration) may be used, e.g., intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the compounds of the invention are formulated in liquid solutions, preferably, in physiologically compatible buffers or solutions, such as saline solution, Hank's solution, or Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms can also be produced.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration, for example, may be through nasal sprays, rectal suppositories, or vaginal suppositories.

For topical administration, the compounds of the invention can be formulated into ointments, salves, gels, or creams, as is generally known in the art. The amounts of various compounds to be administered can be determined by standard procedures taking into account factors such as the compound IC_{50} , EC_{50} , the biological half-life of the compound, the age, size and weight of the patient, and the disease or disorder associated with the patient. The importance of these and other factors to be considered are known to those of ordinary skill in the art.

Amounts administered also depend on the routes of administration and the degree of oral bioavailability. For example, for compounds with low oral bioavailability, relatively higher doses will have to be administered.

Preferably the composition is in unit dosage form. For oral application, for example, a tablet, or capsule may be administered, for nasal application, a metered aerosol dose may be administered, for transdermal application, a topical formulation or patch may be administered and for transmucosal delivery, a buccal patch may be administered. In each case, dosing is such that the patient may administer a single dose.

Each dosage unit for oral administration contains suitably from 0.01 to 500 mg/Kg, and preferably from 0.1 to 50 mg/Kg, of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, calculated as the free base. The daily dosage for parenteral, nasal,

oral inhalation, transmucosal or transdermal routes contains suitably from 0.01 mg to 100 mg/Kg, of a compound of Formula(I). A topical formulation contains suitably 0.01 to 5.0% of a compound of Formula (I). The active ingredient may be administered from 1 to 6 times per day, preferably once, sufficient to exhibit the desired activity, as is readily apparent to one skilled in the art.

As used herein, "treatment" of a disease includes, but is not limited to prevention, retardation and prophylaxis of the disease. As used herein, "diseases" treatable using the present compounds include, but are not limited to leukemias, solid tumor cancers, metastases, soft tissue cancers, brain cancer, esophageal cancer, stomach cancer, pancreatic cancer, liver cancer, lung cancer, bladder cancer, bone cancer, prostate cancer, ovarian cancer, cervical cancer, uterine cancer, testicular cancer, kidney cancer, head cancer and neck cancer, chronic inflammatory proliferative diseases such as psoriasis and rheumatoid arthritis; proliferative cardiovascular diseases such as restenosis; proliferative ocular disorders such as diabetic retinopathy; and benign hyperproliferative diseases such as hemangiomas.

Composition of Formula (I) and their pharmaceutically acceptable salts which are active when given orally can be formulated as syrups, tablets, capsules and lozenges. A syrup formulation will generally consist of a suspension or solution of the compound or salt in a liquid carrier for example, ethanol, peanut oil, olive oil, glycerine or water with a flavoring or coloring agent. Where the composition is in the form of a tablet, any pharmaceutical carrier routinely used for preparing solid formulations may be used. Examples of such carriers include magnesium stearate, terra alba, talc, gelatin, acacia, stearic acid, starch, lactose and sucrose. Where the composition is in the form of a capsule, any routine encapsulation is suitable, for example using the aforementioned carriers in a hard gelatin capsule shell. Where the composition is in the form of a soft gelatin shell capsule any pharmaceutical carrier routinely used for preparing dispersions or suspensions may be considered, for example aqueous gums, celluloses, silicates or oils, and are incorporated in a soft gelatin capsule shell.

Typical parenteral compositions consist of a solution or suspension of a compound or salt in a sterile aqueous or non-aqueous carrier optionally containing a parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil or sesame oil.

Typical compositions for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant such as dichlorodifluoromethane or trichlorofluoromethane.

A typical suppository formulation comprises a compound of Formula (I) or a pharmaceutically acceptable salt thereof which is active when administered in this way, with a binding and/or lubricating agent, for example polymeric glycols, gelatins, cocoa-butter or other low melting vegetable waxes or fats or their synthetic analogs.

- 5 Typical dermal and transdermal formulations comprise a conventional aqueous or non-aqueous vehicle, for example a cream, ointment, lotion or paste or are in the form of a medicated plaster, patch or membrane.

Preferably the composition is in unit dosage form, for example a tablet, capsule or metered aerosol dose, so that the patient may administer a single dose.

- 10 No unacceptable toxicological effects are expected when compounds of the present invention are administered in accordance with the present invention.

The biological activity of the compounds of Formula (I) are demonstrated by the tests indicated hereinbelow.

In vitro assays:

- 15 Compounds capable of inhibiting myt1 kinase can be identified with in vitro assays and cellular assays as described below. Variations of these assays would be obvious to those skilled in the art.

Expression of GST-Myt1:

- 20 A GST-Myt1 expression construct was constructed which has the glutathione-S-transferase gene fused to the amino terminus of Myt1 kinase via a linker containing a thrombin cleavage site. This clone has been truncated at amino acid 362 of Myt1, just prior to the to the transmembrane domain. This construct was cloned into the Baculovirus expression vector, pFASTBAC, and this was used to make the viral stock for the subsequent infection. Spodoptera frugiperda cells (Sf21) were infected with the virus
25 expressing the GST-Myt1 and the cells were grown for 3 days, then harvested and frozen down.

Purification of GST-Myt1:

- 30 The GST-Myt1 protein was purified as follows: An Sf21 cell pellet expressing GST-Myt1 was resuspended on ice in 10mls of lysis buffer (50mM Tris-Cl, pH 7.5, 250mM NaCl₂, 1mM dithiothreitol (DTT), 0.1%NP-40, 5% (v/v) protease inhibitor cocktail, 1mM sodium orthovanadate), cells were lysed by sonication and centrifuged at 100,000xg for 30min The supernatant was added to 5mls (packed volume) of Glutathione Sepharose 4B, equilibrated in wash buffer (20mM Tris-Cl, pH 7.0, 10mM MgCl₂, 100mM NaCl₂, 1mM DTT, 0.5%(v/v) protease inhibitor cocktail, 1mM sodium orthovanadate).

The mixture was rocked for 30min. The resin with the bound GST-Myt1 was spun down at 500xg for 5min and washed with 14mls of wash buffer. The beads were spun as above and resuspended in another 14mls of wash buffer. The suspension was transferred into a column and allowed to pack, then the wash buffer was allowed to flow through by gravity.

- 5 The GST-Myt1 was eluted from the column with 10mls of 10mM Glutathione in 50mM Tris-Cl, pH 8.0 in 500ul fractions. Protein concentrations were determined on the fractions using Bio-Rad's Protein assay kit as per instructions. Fractions containing the GST-Myt1 were pooled and diluted to a concentration of ~0.5mg/ml and dialyzed for 4 hours at 4⁰C in dialysis buffer (20mM HEPES, pH 7.0, 1mM Manganese Acetate, 100mM NaCl₂, 0.05% Brij-35, 10% glycerol, 1mM DTT, 0.2% (v/v) protease inhibitor cocktail, 1mM sodium orthovanadate). The protein was aliquoted and stored at -80⁰.
- 10

Enzyme Assays:

GST-Myt1 autophosphorylation-DELFI A assay

- Delayed fluorescent immunoassays (DELFI A) were performed in 96well NUNC maxisorp plates, at 50ul/well with 0.25ug GST-Myt1, in BufferA (50mM HEPES, pH 7.4, 2mM Mn(OAc)₂, 5uM ATP, 1mM DTT). (DELETE) For determination of pH optimum, divalent cation usage and K_m of ATP, the appropriate component was varied as indicated in the figures. (DELETE) Autophosphorylation reactions were initiated by the addition of GST-Myt1 in buffer and were allowed to proceed at room temperature with shaking for
- 20 20min. The reactions were stopped with the addition of EDTA to a 20mM final concentration, and the protein was allowed to continue to bind to the wells for an additional 40min. Wells were washed three times with 300ul TBS/Tween (50mM Tris, pH 7.4, 150mM NaCl₂, 0.2% Tween-20). After washing, the plate was blocked using Pierce's Superblock in TBS at 100ul/well. This was immediately decanted and the blocking was
- 25 repeated two more times. The plate was then washed again with three washes of 300ul/well of TBS-Tween. Then 100ul of Eu-labeled anti-phosphotyrosine antibody diluted to 0.125ug/ml in TBS/Tween containing 0.15mg/ml BSA was added to the wells and allowed to incubate for 30min with shaking at room temperature. Wells were then washed three times with 300ul of TBS/Tween, 200ul of Enhancement solution was added per well and
- 30 incubated with shaking for 10min. The plate was then read on the 1420 VICTOR plate counter from Wallac, Inc. The identical conditions are used for inhibitor studies except that ATP is at 1uM and inhibitors are added, in dimethyl sulfoxide (DMSO) to a final concentration of 1%. Typical concentration ranges in

which test compounds are expected to inhibit myt1 autophosphorylation are 0.001 to 10 uM.

Biological Studies:

Cell Cycle Studies

- 5 Drug studies considering cellular effects were performed in the Hela S3 adherent cell line. Cells were plated at a concentration sufficiently low, such that 24 hours later they were at 10-20% confluence (typically 2×10^5 cells/15cm²). Cells were then synchronized in S phase by a repeated thymidine block. Briefly, cells were treated with 2mM thymidine for 18 hours, released for 8 hours by 3 washes, and then treated again with thymidine.
- 10 Following the second release from thymidine, 95% of cells were in S phase. Synchronized cells were then returned to complete media containing a DNA-damaging drug such as 50nM topotecan (a dosage we have found to be sufficient to arrest cells in early G2 phase without inducing apoptosis) alone and in combination with test compounds for up to 18 hours. Cell Cycle profiles were then performed cytometrically using a procedure for
- 15 propidium iodide staining of nuclei. (Vindelov et al, Cytometry Vol.3, No.5, 1983, 323-327) Myt1 inhibitors would be expected to reverse the G2 arrest caused by the DNA damaging agent. Typical concentration ranges for such activity would be 0.001 to 10 uM.

Proliferation/Apoptosis Studies:

- 20 Proliferation studies were performed in a variety of adherent and non-adherent cell lines including Hela S3, HT29, and Jurkat(delete ***). The proliferation assay utilized a colorimetric change resulting from reduction of the tetrazolium reagent XTT into a formazan product by metabolically active cells (Scudiero et al. Cancer Research, 48, 1981, 4827-4833) Cells were seeded in 100uls in 96 well plates to roughly 10% confluence (cell concentration varied with cell lines) and grown for 24 hours. Compounds were then added
- 25 with or without sufficient vehicle- containing media to raise the cells to a 200ul final volume containing chemical reagents in 0.2% DMSO. Cells received multiple concentrations of DNA-damaging anti-proliferative drugs such as topotecan, test compounds, and combination treatment at 37°C 5% CO₂. 72 hours later, 50 uls of an XTT/phenazine methosulfate mixture were added to each well and cells were left to incubate for
- 30 90mins. Plate was read at 450nm, and anti-proliferative effects were compared relative to vehicle treated cells. Myt1 inhibitors are expected to inhibit the proliferation of such cancer cell lines and/or enhance the cytotoxicity of DNA-damaging chemotherapeutic drugs. Typical concentration ranges for such activity would be 0.001 to 10 uM. Other assays for

cellular proliferation or cytotoxicity could also be used with test compounds, and these assays are known to those skilled in the art.

The present invention includes but is not limited to the examples below. Nuclear magnetic resonance spectra were recorded at 300 MHz using a Bruker AM 300 spectrometer. CDCl₃ is deuteriochloroform, DMSO-d₆ is hexadeuteriodimethylsulfoxide, and CD₃OD is tetradeuteriomethanol. Chemical shifts are reported in parts per million (?) downfield from the internal standard tetramethylsilane. Abbreviations for NMR data are as follows: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, dd=doublet of doublets, dt=doublet of triplets, app=apparent, br=broad. J indicates the NMR coupling constant measured in Hertz. Continuous wave infrared (IR) spectra were recorded on a Perkin-Elmer 683 infrared spectrometer, and Fourier transform infrared (FTIR) spectra were recorded on a Nicolet Impact 400 D infrared spectrometer. IR and FTIR spectra were recorded in transmission mode, and band positions are reported in inverse wavenumbers (cm⁻¹). Mass spectra were taken on either VG 70 FE, PE Syx API III, or VG ZAB HF instruments, using fast atom bombardment (FAB) or electrospray (ES) ionization techniques. Elemental analyses were obtained using a Perkin-Elmer 240C elemental analyzer. Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. All temperatures are reported in degrees Celsius.

Analtech Silica Gel GF and E. Merck Silica Gel 60 F-254 thin layer plates were used for thin layer chromatography. Both flash and gravity chromatography were carried out on E. Merck Kieselgel 60 (230-400 mesh) silica gel. Analytical and preparative HPLC were carried out on Rainin or Beckman chromatographs. ODS refers to an octadecylsilyl derivatized silica gel chromatographic support. 5 μ Apex-ODS indicates an octadecylsilyl derivatized silica gel chromatographic support having a nominal particle size of 5 μ, made by Jones Chromatography, Littleton, Colorado. YMC ODS-AQ® is an ODS chromatographic support and is a registered trademark of YMC Co. Ltd., Kyoto, Japan. PRP-1® is a polymeric (styrene-divinylbenzene) chromatographic support, and is a registered trademark of Hamilton Co., Reno, Nevada) Celite® is a filter aid composed of acid-washed diatomaceous silica, and is a registered trademark of Manville Corp., Denver, Colorado.

Example 1

bis[2-(4-phenyl-5-methyl)thiazolyl]amine hydrobromide

- MS (ES) m/e 363.7 [M+H]⁺ ¹H NMR (300MHz, DMSO-d) ??7.74(d, J=10Hz, 4H), 7.52(t, J=6Hz, 4H), 7.39(t, J=11Hz, 2H).

Example 2

bis[2-(4-(2-pyridyl)thiazolyl]amine dihydrobromide

- MS (ES) m/e 337.7 [M+H]⁺ ¹H NMR (300MHz, DMSO-d) ??8.71(d, J=3Hz, 2H), 8.23(m, 4H), 8.05(s, 2H), 7.59(t, J=6Hz, 2H)

Example 3

bis[2-[4-(3-pyridyl)thiazolyl]amine bistrifluoroacetate

- MS (ES) m/e 337.7 [M+H]⁺ ¹H NMR (300MHz, DMSO-d) ??9.33(s, 2H), 9.04(d, J=10Hz, 2H), 8.75(d, J=5Hz, 2H), 8.08(t, J=11Hz, 2H), 7.88(s, 2H)

Example 4

N,N-Bis(5(3,4-dichlorophenyl)-2-thiazolyl)amine dihydrobromide

- ¹H NMR (300MHz, DMSO-d) ??8.33 (s, 1H), 8.18 (s, 2H), 7.94 (d, J=8.2Hz, 2H), 7.82 (s, 2H), 7.73 (d, J=8.2Hz, 2H).

Example 5

1,3-bis[4-(3-pyridyl)-2-thiazolylamino]benzene dihydrobromide

- MS (ES) m/e 428.8 [M+H]⁺ ¹H NMR (300MHz, DMSO-d) ??10.44 (s, 2H), 9.17 (d, J=1.7 Hz, 2H), 8.47 (dd, J=1.7, 4.7Hz, 2H), 8.23-8.29 (m, 4H), 7.56(s, 2H), 7.39 (dd, J=5.0, 8.0 Hz), 7.32 (s, 2H),

Formulations for pharmaceutical use incorporating compounds of the present invention can be prepared in various forms and with numerous excipients. Examples of such formulations are given below:

Example 6**Inhalant Formulation:**

A compound of Formula (I), (1 mg to 100 mg) is aerosolized from a metered dose inhaler to deliver the desired amount of drug per use.

5

Example 7**Tablet Formulation:**

Tablets/Ingredients	Per Tablet
1. Active ingredient	40 mg
10 (Cpd of Form. (I))	
2. Corn Starch	20 mg
3. Alginic acid	20 mg
4. Sodium Alginate	20 mg
5. Mg stearate	1.3 mg

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Procedure for Tablet Formulation:

Ingredients 1, 2, 3 and 4 are blended in a suitable mixer/blender. Sufficient water is added portion-wise to the blend with careful mixing after each addition until the mass is of a consistency to permit its conversion to wet granules. The wet mass is converted to granules by passing it through an oscillating granulator using a No. 8 mesh (2.38 mm) screen. The wet granules are then dried in an oven at 140°F (60 °C) until dry. The dry granules are lubricated with ingredient No. 5, and the lubricated granules are compressed on a suitable tablet press.

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Example 8**Parenteral Formulation**

A pharmaceutical composition for parenteral administration is prepared by dissolving an appropriate amount of a compound of Formula I in polyethylene glycol with heating. This solution is then diluted with water for injections (to 100 mL). The solution is then rendered sterile by filtration through a 0.22 micron membrane filter and sealed in sterile containers.

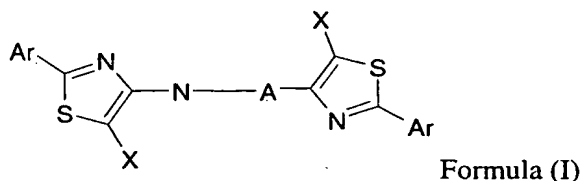
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All publications, including but not limited to patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication

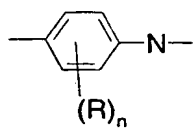
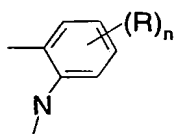
were specifically and individually indicated to be incorporated by reference as though fully set forth.

What is claimed is:

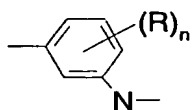
1. A compound according to Formula (I), hereinbelow:



wherein A represents a covalent bond, or a 1,2, 1,3 or 1,4-disubstituted aryl amine ring selected from the group consisting of:



, and



wherein

R is independently selected from the group consisting of H, OMe, Cl, Br, F, NO₂, and CN;

n is an integer from 1 to 4;

each X is independently selected from the group consisting of H, Br, CH₃, NO₂, CN, and

NR₁R₂;

each Ar is independently optionally substituted phenyl or an optionally substituted 5 or 6 membered heterocyclic ring comprising one or more heteroatoms selected from N, O and S; and

R₁ and R₂ are, independently, hydrogen or C₁₋₄ alkyl, branched or cyclic, optionally

containing O or N.

2. A compound according to claim 1 selected from the group consisting of:

bis[2-(4-phenyl-5-methyl)thiazolyl]amine,

bis[2-(4-(2-pyridyl)thiazolyl]amine,

bis[2-[4-(3-pyridyl)thiazolyl]amine,

- N,N-Bis(5(3,4-dichlorophenyl)-2-thiazolyl)amine,
N,N-bis(4-(4'-biphenyl)-2-thiazolyl)amine,
1,4-bis(4-(4'-biphenyl)-2-thiazolylamino)benzene,
1,3-bis[4-(3-pyridyl)-2-thiazolylamino]benzene,
5 1,4-bis[4-fluorophenyl-2-thiazolylamino]benzene; and
1,4-bis(4-(4-methoxyphenyl)-2-thiazolylamino)benzene.
3. A compound according to claim 2 selected from the group consisting of:
bis[2-(4-phenyl-5-methyl)thiazolyl]amine,
1,4-bis(4-(4-methoxyphenyl)-2-thiazolylamino)benzene,
10 N,N-bis(4-(4'-biphenyl)-2-thiazolyl)amine,
1,4-bis(4-(4'-biphenyl)-2-thiazolylamino)benzene,
1,4-bis[4-fluorophenyl-2-thiazolylamino]benzene,
bis[2-(4-(2-pyridyl)thiazolyl)amine and
bis[2-[4-(3-pyridyl)thiazolyl)amine.
- 15 4. A compound according to claim 3 selected from the group consisting of:
bis[2-(4-phenyl-5-methyl)thiazolyl]amine,
1,4-bis(4-(4-methoxyphenyl)-2-thiazolylamino)benzene,
N,N-bis(4-(4'-biphenyl)-2-thiazolyl)amine, and
1,4-bis(4-(4'-biphenyl)-2-thiazolylamino)benzene hydrobromide.
- 20 5. A method of antagonizing a myt1 kinase receptor which comprises administering to
a subject in need thereof, an effective amount of a compound according to claim 1.
6. A method of treating a disease or disorder selected from the group consisting of
leukemias, solid tumor cancers and metastases, soft tissue cancers, brain cancer, esophageal
cancer, stomach cancer, pancreatic cancer, liver cancer, lung cancer, bladder cancer, bone
25 cancer, prostate cancer, ovarian cancer, cervical cancer, uterine cancer, testicular cancer,
kidney cancer, head cancer and neck cancer, chronic inflammatory proliferative diseases,
proliferative cardiovascular diseases, proliferative ocular disorders and benign
hyperproliferative diseases which comprises administering to a subject in need thereof an
effective amount of a compound according to claim 1.
- 30 7. A method according to claim 4 wherein the disease or disorder treated is selected
from the group consisting of psoriasis, rheumatoid arthritis, diabetic retinopathy and
hemangiomas.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/28989

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/4406; C07D 277/38, 417/04, 417/10

US CL :514/342, 377; 546/269.7; 548/190

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/342, 377; 546/269.7; 548/190

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BERNDT et al. Some Reactions of 2-Benzimidazolecarbonitrile. J. Heterocycl. Chem. February 1972, Vol. 9, No. 1, pages 137-140. See entire document also the attached abstract.	1-7



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 MARCH 2000

Date of mailing of the international search report

06 APR 2000

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